1513-Pos Board B243
Modified Lipid Content Affects Daptomycin-Membrane Interactions
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Daptomycin is an anionic membrane active antimicrobial lipopeptide used to treat serious infections caused by gram-positive bacteria. It causes target membrane depolarization by forming oligomeric pores that allow leakage of potassium ions, but the complete mechanism of action is unresolved. Antibiotic function is calcium dependant and requires the presence of anionic phospholipids like phosphatidylglycerol (PG) in the target membrane. Because of its efficacy in treating infections resistant to many front-line antibiotics, the recent emergence of strains displaying reduced daptomycin susceptibility is troubling. These quasi-resistant strains have modified membrane lipid content, including an increased presence of cardiolipin (CL) and lysyl-PG, the latter possibly due to the observed up-regulation of mprF, a gene that codes for a protein with lysyl-PG synthase and flippase activity. Using monolayer and bilayer model systems, we sought here to study the effects of the presence of CL on daptomycin binding to PG-containing membranes. Surprisingly, isothermal titration calorimetry (ITC) revealed that daptomycin-membrane affinity increased when small amounts of CL was present and continued to increase until 10 mol%, above which the trend reversed drastically. Results from Langmuir monolayer insertion experiments also show that CL affects the degree of drug insertion into PG-containing lipid films as determined by greater increases in surface pressure after daptomycin was injected into the aqueous subphase when CL was present. Preliminary results from monolayer insertion experiments in which lysyl-PG was included in the films indicate that this lipid also significantly affects drug-monolayer interactions. We hypothesize from these results that alteration of the lipid content of bacterial membranes represents an important component of the potential resistance mechanism and should be considered in the development and formulation of the next generation of membrane targeting antibiotics.

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Membrane-Dependent Activity of the Dermcidin Channel
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Dermcidin (DCD) is a peptide found in human sweat which exerts an antimicrobial activity by pore formation in bacterial membranes. Structural studies show that a channel-like assembly is formed by a hexamer of the α-helical peptide. In order to understand how differences in membrane lipid composition may explain the selectivity of the antimicrobial activity of DCD, we performed coarse-grained molecular dynamics simulations to study the orientation of the hexameric channel structure of DCD in various lipid bilayers. Our results indicate that, the DCD channel exhibits a tilted orientation when embedded in lipid bilayers, and the tilt angle depends on the thickness of the bilayer. The thicker the bilayer, the less the tilt. The presence of cholesterol, which thickens the bilayer, also reduces the tilt angle. Therefore, we believe the hydrophobic mismatch plays a key role in determining the orientation of the DCD oligomer in membranes. Also, all-atom molecular dynamics simulations were performed to investigate the conductance of the channel with different orientations. We believe that the tilt angle of DCD may be related to its antimicrobial activity, probably by modulating the ion/water permeability through the channel.

1515-Pos Board B245
Modelling the Interactions of Equinatoxin II with Micelles
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Equinatoxin II (EqtII) is a 179-residue toxin from the venom of the sea anemone Actinia equina. EqtII is a member of the actinoporin family of proteins, which have potent lytic activity towards membranes containing sphingomyelin (SM). To gain insight into the atomic-level details governing SM selectivity, a series of all-atom molecular dynamics simulations were performed to model the binding of EqtII to micelles of n-dodecylphosphocholine (DPC) and DPC/SM. These models are in good agreement with concurrent high-resolution solution NMR studies and prior data [1, 2] that suggests membrane binding is dependent on a conserved cluster of aromatic amino acids. From this groundwork study, further simulations will be performed to investigate EqtII oligomerisation and membrane insertion to determine the mechanism of pore formation.
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1516-Pos Board B246
Partitioning Charged Side Chains into Lipid Bilayer Membranes
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Hydrophobic amino acids are abundant in transmembrane (TM) helices of membrane proteins. Charged residues are sparse, apparently due to the unfavorable energetic cost of partitioning charges into non-polar phases. Nevertheless, conserved arginine residues within TM helices regulate vital functions, such as ion channel voltage gating and integrin receptor inactivation. The energetic cost of arginine in various positions along hydrophobic helices has been controversial. Potential of mean force (PMF) calculations from atomistic molecular dynamics simulations predict very large energetic penalties, while in vitro experiments with Sec61 translocons indicate much smaller penalties, even for arginine in the center of hydrophobic TM helices. Resolution of this conflict has proved difficult, because the in vitro assay utilizes the complex Sec61 translocon, while the PMF calculations rely on the choice of simulation system and reaction coordinate. Here we present the results of computational and experimental studies that permit direct comparison with the Sec61 translocon results. We find that arginine can be accommodated at the center of a hydrophobic TM helix without a significant energetic penalty for peptides designed using templates from the in vitro Sec61 experiments. Furthermore, the translocon assay seems to underestimate the probability of insertion compared to our computational results. We show that a combination of arginine snorkeling, bilayer deformation, and peptide tilting is sufficient to lower the penalty of arginine insertion, thus providing an explanation for the differences between PMF- and experiment-based burial penalties.
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Membrane Translocation of Highly Charged Antimicrobial Peptides via Multi-Microsecond All-Atom Md Simulations
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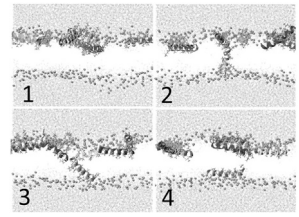
We report the first computational description of the membrane translocation mechanism and pathway of two antimicrobial peptides, PGLa from X. laevis (charge +5), and maculatin from L. genimaculata (charge +2), via multi-microsecond MD simulations. At P/L=1:20-50, assembly of monomers into higher oligomeric aggregates, or the formation of pores, is not observed for either peptide. Instead, individual monomers are transported across the bilayer in a two-stage mechanism in which charged side-chains are separately translocated via assistance of other peptides. Transmembrane inserted water-free intermediate states are observed that are stable over many microseconds. Our proposed mechanism disagrees with earlier models of antimicrobial bilayer penetration, and suggests that translocation of highly charged peptides across a membrane is simpler than previously thought.



Protein-Lipid Interactions II

1518-Pos Board B248
Ion Transport and Electrochemical Gradients under DC and AC Signals
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The cell must be able to exchange materials with its surrounding environment *via* the plasma membrane in order to survive. Here we investigate passive ion